Examiner: Shukla, R. Our Docket No.: 24601-4021 Art Unit: 1632 MAR 1 3 2003 TRANSFECTION **CHROMATIDS** CENTROMERE KINETOCHORE **TELOMERE** ACM8 AND AgtWESneo DNA INTEGRATION TELOMERE В 3. 34 W. 5. W. **EUCHROMATIN** HETEROCHROMATIN (SATELLITE DNA) MOUSE CHROMOSOME (LMTK "CELL LINE) AMPLIFICATION CENTROMERE FORMATION neo-CENTROMERE 12 3 14 1 1 1 1 1 1 1 SELECTION (G-418) D TELOMERIC DNA KINETOCHORE SATELLITE DNA MITOTIC SPINDLE DICENTRIC CHROMOSOME (EC3/7 CELL LINE) CHROMOSOME BREAKAGE "FOREIGN" DNA SATELLITE DNA neo-CENTROMERE TELOMERE FORMERLY DICENTRIC CHROMOSOME CHROMOSOME FRAGMENT WITH neo-CENTROMERE 10-15Mb (EC3/7 CELL LINE) DUPLICATION-SINGLE CELL CLONING G reo-CENTROMERE TELOMERE TELOMERE/mCPE 1.51 SATELLITE DNA SATELLITE DNA "FOREIGN"DNA 20-30Mb neo-MINICHROMOSOME (EC3/7-C5;EC3/7-C6 CELL LINES)

HELLER EHRMAÑ WHII E & MICAULIFFE LLP Sheet 1 of 5 e: ARTIFICIAL CHROMOSOMES, USES THEREOF AND THODS FOR PREPARING ARTIFICIAL CHROMOSOMES

Filed: April 17, 2001 ..

Serial No.: 09/836,911

FIG. I

TECH CENTER 1600/290

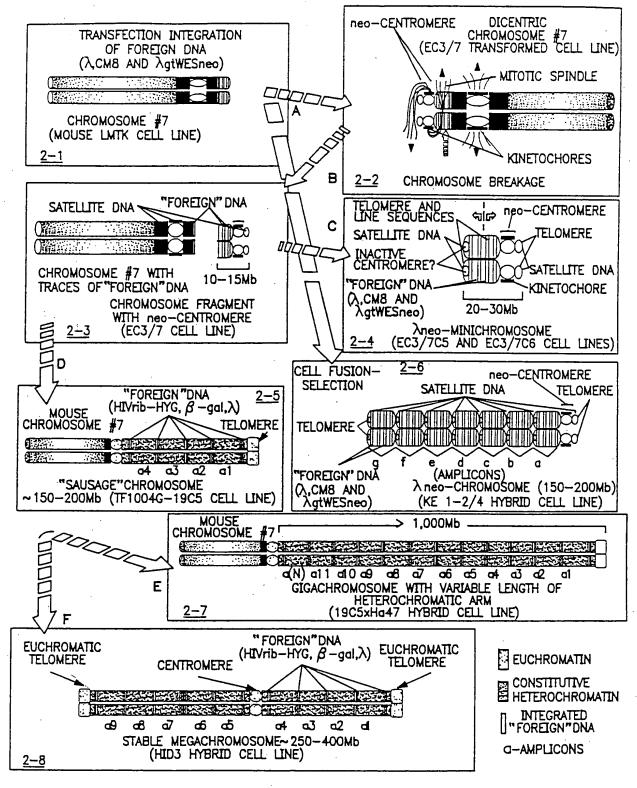


FIG. 2

Applicant: Hadlaczky et al. Serial No.: 09/836,911 Filed: April 17, 2001

Examiner: Shukla, R. Our Docket No.: 24601-4021

TECH CENTER 1600/2900

PRIMARY REPLICATION INITIATION SITE (MEGAREPLICATOR)

SECONDARY ORIGINS OF REPLICATION

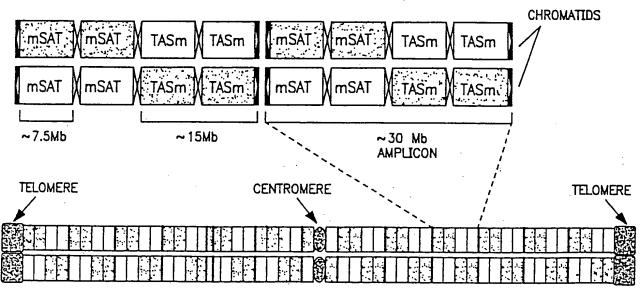
MEGAREPLICON OF THE CENTROMERIC REGION OF MOUSE CHROMOSOMES WITH TWO~7.5Mb TANDEM BLOCKS OF MOUSE MAJOR SATELLITE DNA (mSAT) FLANKED BY NON-SATELLITE DNA SEQUENCES

INTEGRATION OF "FOREIGN"DNA (pH132, pCH110,λ)

REPLICATION ERROR GENERATES INVERTED MEGAREPLICONS



AMPLIFICATION PRODUCES A TANDEM ARRAY OF IDENTICAL CHROMOSOME SEGMENTS (AMPLICONS) THAT CONTAIN TWO INVERTED MEGAREPLICONS BORDERED BY THE HETEROLOGOUS ("FOREIGN") DNA



STABLE MEGACHROMOSOME (~250-400Mb)

FIG. 3

HELLER EHRMAN WHILL & MCAULIFFE LLP

Serial No.: 09/836,911 Examiner: Shukla, R. OUR DOCKET NO.: 24601-4021

Filed: April 17, 2001 .

EC3/7

MOUSE LMTK" (FIBROBLAST CELL LINE WITH neo-CENTROMERE)

SINGLE-CELL SUBCLONING

FUSION WITH CHO K20 CELLS AND 8 ▼ SELECTION WITH G418 AND HAT

EC3/7C5

MOUSE LMTK FIBROBLAST CELL LINE WITH THE neo-MINICHROMOSOME AND THE FORMERLY DICENTRIC CHROMOSOME

KE1 - 2/4

MOUSE-HAMSTER HYBRID CELL LINE WITH THE STABLE Aneo-CHROMOSOME

COTRANSFECTION WITH PLASMIDS pH132 (ANTI-HIV RIBOZYME AND HYGROMYCIN-RESISTANCE GENES), pCH110 (lacZ GENE) AND λ cl 875 Sam7 (λPHAGE), SELECTION WITH HYGROMYCIN B

TF1004G-19C5

MOUSE LMTK FIBROBLAST CELL LINE WITH neo-MINICHROMSOME AND STABLE SAUSAGE CHROMOSOME

- FUSION WITH CHINESE HAMSTER OVARY CELLS (CHO K20 CELL LINE), SELECTION WITH HAT AND HYGROMYCIN B.

19C5xHa4,

19C5xHa3 --- RECLONING --- 19C5xHa47

(CARRIES THE GIGACHROMSOME)

MOUSE-HAMSTER HYBRID CELL LINES CARRYING THE neo-MINICHROMOSOME AND THE SAUSAGE CHROMOSOME AND COUNTAINING A COMPLETE HAMSTER GENOME AND PARTIAL MOUSE GENOME

<u>G3D5</u>

AND neo-

BrdU TREATMENT, SINGLE-CELL CLONING, SELECTION WITH HYGROMYCIN B

BrdU TREATMENT. SINGLE-CELL CLONING, SELECTION WITH G418, BrdU TREATMENT AND RECLONING

RECLONE AND GROW

H₁D₃

MOUSE-HAMSTER HYBRID CELL LINE CARRYING A MEGACHROMOSOME BUT NO MINICHROMOSOME

FUSION WITH CD4+ HeLa CELLS CONTAINING neor. SELECTION WITH G418 AND HYGROMYCIN B

H1xHe41

MOUSE-HAMSTER-HUMAN HYBRID CELL LINE CARRYING THE MEGACHROMOSOME AND A SINGLE HUMAN CHROMOSOME WITH CD4 AND

GHB42 CARRIES MEGA-CHROMOSOME AND neo-MINICHROMOSOME

IN G418 AND

▼ HYGROMYCIN B

MEGACHROMOSOME

MINICHROMOSOME

neo- MINICHROMOSOME ONLY RECLONE AND GROW IN G418

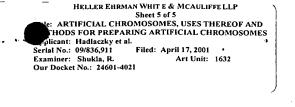
<u>GB43</u>

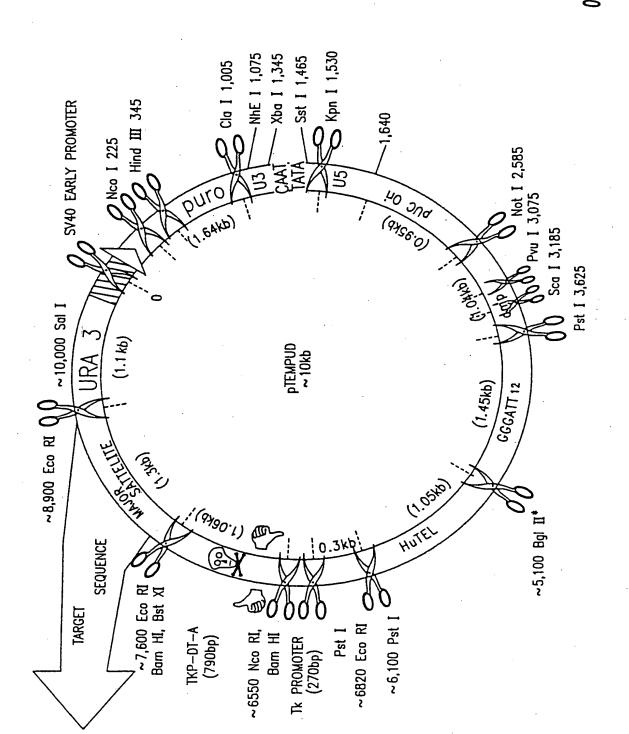
G3D6

MOUSE-HAMSTER HYBRID CELL LINES CARRYING:

CARRIES neo-MINICHROMOSOME ONLY

neor GENES; CONTAINS COMPLETE HAMSTER AND PARTIAL MOUSE GENOMES





-1G. 5